Combined chlorophyll fluorescence techniques to study environmental impact on the mountain moss *Polytrichum commune*

Gabriella Nora Maria Giudici

University of Naples Federico II, Department of Agriculture, 80055 Portici, Italy

Abstract

Two chlorophyll fluorescence (ChlF) methods were used to study the effects of high light (photoinhibition) and dehydration, common stressors of the alpine environment, on primary photosynthetic processes in the moss *Polytrichum commune* from the Czech Republic, the Jeseníky Mountains. Photoinhibition (PI) was studied in fully hydrated thalli of *P. commune* and during the period of spontaneous desiccation. Time courses of Kautsky kinetics (KK) of ChlF and derived parameters: maximum quantum yield (F_V/F_M), effective quantum yeld (Φ_{PSII}), and non-photochemical quenching parameters, were measured before and after the samples were treated with high light (1500 μmol m^{-2} s^{-1} PAR) for 60 min. Dehydration effects were tested in two sets of experiments with a Pulse-Amplitude-Modulation fluorometry (PAM) and Fast Chlorophyll Fluorescence induction curve (OJIP) techniques. In PAM tests, the desiccating samples were exposed to saturating light pulses every 10 min. in order to obtain Φ_{PSII} and non-photochemical quenching (NPQ). In the second dehydration experiment, OJIP transients of ChlF were repeatedly recorded, OJIP-derived ChlF parameters were plotted against relative water content (RWC) monitored during desiccation. Combined ChF techniques provided insights into the mechanisms activated during *P. commune* desiccation, such as dissipation of excess absorbed energy through heat dissipation, and conformational changes or destructions of the light harvesting complexes. Combination of stressors resulted in amplified interference with the photosynthetic machinery, even when the added stressor (dehydration) was applied in low dose.

Key words: OJIP, PAM, Kautsky Kinetics, *Polytrichum*, alpine environment, photoinhibition, desiccation

Introduction

Mosses from sunny habitats are considered tolerant or resistant to photoinhibition due to several photoprotective mechanisms (*see* below). Some of these are also activated in desiccating mosses (*e.g.* Csintalan et al. 1999, Nabe et al. 2007), as a part of non-photochemical quenching mechanisms.

The two factors, photoinhibition and moss desiccation typically co-act in the field. Some recent studies, however, have focused on the sensitivity of mosses to photoinhibition in the optimally hydrated state (*e.g.* Orekhova et al. 2021a). In the field, air/thallus temperature affect the moss pho-

---

tosynthesis as well. These effects were studied by Perera-Castro et al. (2020) who combined chlorophyll fluorescence (ChlF) and gas exchange measurents. They evaluated temperature optima for photosynthesis in six Antarctic mosses. They reported a high temperature-induced increase in NPQ. However, they did not consider photoinhibition.

This study on *Polytrichum commune*, therefore, focuses on time courses of chlorophyll fluorescence (ChlF) parameters (F_v/F_M, Φ_{PSII}, NPQ) during controlled photoinhibition and desiccation. It was hypothesized that the resistance of *P. commune* to photoinhibition/desiccation would be high. Therefore, the activation of photoprotective mechanisms during controlled photoinhibitory/desiccation treatment was expected. Special attention was devoted to non-photochemical quenching that reflects the activation of protective mechanisms (Beckett et al. 2005).

Photoprotective mechanisms comprise cyclic electron transport around photosystem I (PSI) and photorespiration (Takahashi and Badger 2011). Another mechanism relates to carotenoids that scavenge ROS. Carotenoids are also involved in thermal dissipation (part of non-photochemical quenching). Photoinhibition-induced formation of zeaxanthin and consequent energy quenching is reported in mosses (Pinnola et al. 2013, Glime 2017). It is established that non-photochemical quenching in mosses is dependent on desiccation and photoinhibition (Heber et al. 2006). Recently, three different components of non-photochemical quenching are reported (reviewed in *e.g.* Yamakawa et al. 2012): (i) a mechanism based on protonation of a thylakoid protein (active in hydrated mosses); (ii) exciton migration towards the light-harvesting complexes (LHCs), where fast thermal dissipation takes place, and (iii) reversible photo-accumulation of radicals that acts as a quencher of excitation energy in RC of PSII.

Polytrichum commune is a moss inhabiting a wide range of habitats. It is considered almost cosmopolitan species, known from all continents except Antarctica. In Europe, it grows on acid soil in moist places. Typically, it is abundant in coniferous forests, wet meadows, on the edges of peat bogs. For the experiment, *P. commune* from alpine environment of the Jeseníky Mountains, Czech Republic, was selected. Environmental conditions such as desiccation and high light stress are the factors potentially dangerous to the species survival, especially in a global climate change scenario. In this study, the efficiency of primary photosynthetic processes was investigated using two different chlorophyll fluorescence techniques, in order to evaluate *P. commune* response to high light (*i.e.* inducing photoinhibition) or desiccation in single or combined treatments.

Material and Methods

Collection of samples and species characteristics

Polytrichum commune was collected in a treeless alpine area in the Jeseniky Mountains, close to the Tabulové skály rocks, (1 415 m a. s. l.), Moravian-Silesian region of Czech Republic. After collection, the samples were stored in the lab at 5°C, under dim light (20-25 µmol m^{-2} s^{-1} PPFD),

with the lower part of the shoots in water and the upper part in fully wet conditions.

P. commune, a poikilohydric organism, has a typical leaf structure to adjust to negative environment conditions. The cross section of a single leaf has a cuplike structure with chloroplasts inside single-lined

cells, arranged in parallel string structures called *lamellae*, packed inside this cuplike shape (Fig. 1). When dehydration occurs, the margins of the leaf fold towards the middle line, closing the structure of lamellae and reducing water exchange between internal structure and environment. In this way, the leaf is able to reduce water loss and retain physiological activity. These movements can be observed even for low level of dehydration, making any kind of ChlF measurements affected because of the changing geometry of leaves and the consequent effect on ChlF readings.
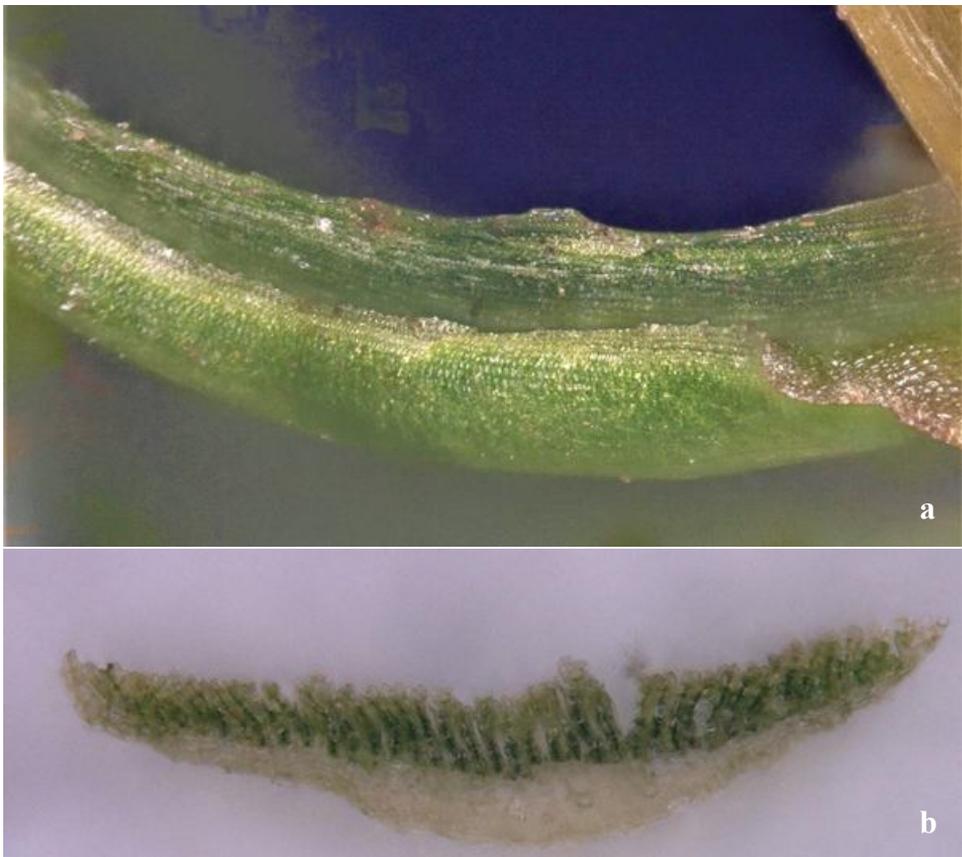

Fig. 1. *Polytrichum commune* single leaf half folded inward (a) and microscopy section of a single leaf showing the lamellae arranged in the cuplike shape perfectly unfolded (b).

Experimental design

Desiccation was studied by the PAM technique (PAM-2000 fluorometer, Walz, Germany) and the OJIP technique (FluorPen FP 100, Photon System Instruments, Czech Republic). The PAM technique comprised of the measurements of slow Kautsky kinetics (KKs) supplemented with saturation pulses in dark- and light-adapted states of the sample (for more details *see e.g.* Marečková et al. 2019). The chlorophyll fluorescence (KKs and the ChlF parameters, *see* below) were recorded us-

ing a FluorCam HFC 1000-H (Photon Systems Instruments, Drásov, Czech Republic) and the FluorCam v. 7.0 software. The following parameters were calculated: F_V/F_M, Φ_{PSII}, NPQ.

Photoinhibition was studied in fully wet conditions and during spontaneous desiccation conditions by Kautsky kinetics techniques (Handy Fluorcam HFC-010, Photon Systems Instruments, Czech Republic).

Photoinhibition

Fully hydrated samples (with their shoots in water and kept in fully wet conditions by continuous spraying with distilled water), along with the samples left to spontaneous water loss, were treated with light of 1 500 μmol m^{-2} s^{-1} PAR for 60 min. Measurements of Kautsky kinetic transients (five replicates) were made immediately before, immediately after and every 20 min. for 3 h after the treatment. ChlF parameters (maximum quantum yield - F_V/F_M, effective quantum yield - Φ_{PSII}, and non-photochemical quenching parameters) were plotted as time courses. The samples were darkened for 5 min. before each measurement. Each sample of the set left to spontaneous desiccation was weighted be-

fore every measurement, from fully wet conditions, to the end of the experiment, and after complete desiccation (in oven at 90°C for 72 h), to assess the relative water content (RWC) corresponding to each measurement. The RWC was calculated by the formula:

$$RWC\,(\%) = [(FM - DM)/(FW - DM)] * 100$$

where, FM is the weight of the fresh sample, DM is the weight of the fully dry sample and FW is the weight of the fully wet sample. So, in fully wet sample RWC = 100%, in fully desiccated sample RWC = 0%.

Desiccation (KK measurements)

Samples, previously acclimated for 10 min. to dim light (20 μmol m^{-2} s^{-1} PAR), were weighted in their fully wet conditions and put on a scale continuously measuring their weights. Over them, very close to their surfaces, the probe of the PAM fluorometer was providing saturating

light pulses every 10 min. and recording ChlF parameters. Each record was related to the weight taken at the very moment of the pulse in order to relate the ChlF parameters change to the dehydration curve expressed as RWC.

Desiccation (OJIP measurements)

Samples were weighted in their fully wet conditions and OJIP transients were repeatedly measured along with their weights during desiccation in lab conditions (22°C±1°), until the signal was too low for measurement. Before every OJIP measurements, the samples were darkened for 5 min. and, after the measurements, the light was kept under 40 μmol m^{-2} s^{-1} PAR.

Recorded transients of each sample were related to the RWC at the time of recording, ChlF parameters were plotted as response curves to RWC decrease. OJIP measurements provided a large amount of data, below there is a list of some used in the study, with calculation formulas and description (adopted from Strasser et al. 2004).

F_V/F_M	Maximal quantum yield of PSII photochemistry
$ABS/RC = M_0(1/V_J)(1/Phi\_P_0)$	Absorption flux (of antenna chlorophylls) per Reaction Center (RC)
$TR_0/RC = M_0(1/V_J)$	Trapped energy flux (leading to chinon A reduction) per RC
$ET_0/RC = M_0(1/V_J)Psi\_E_0$	Electron transport flux (further than chinon A) per RC
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	The flux of dissipated excitation energy at time 0
$PI\_tot = Pi\_ABS\,[(RE_0/TE_0)/(1 - RE_0/TE_0)],$ *where* $RE_0/TE_0 = (F_M - F_I)/(F_M - F_J)$	Total performance index on absorption basis for energy conservation from exciton to the reduction of photosystem I acceptors

Table 1. OJIP-derived parameters and their formulas according to Strasser et al. (2004).

Results

Photoinhibition

F_V/F_M and Φ_{PSII} showed photoinhibitory treatment-dependent decrease (Fig. 2) followed by a recovery, which was faster and more completed in F_V/F_M (above 93% of pre-photoinhibitory values) than Φ_{PSII} (about 91%). In the samples desiccating during the photoinhibitory treatment, decrease of F_V/F_M was more pronounced than in wet samples. The rate of recovery was significantly slower in desiccating than wet samples. However, at the end of recovery period (3 h), F_V/F_M reached the same values for wet and desiccating samples.

Similar response was found for Φ_{PSII}, *i.e.* more pronounced Φ_{PSII} decrease in desiccating samples exposed to photoinhibitory treatment. More uncompleted recovery and lower Φ_{PSII} values were found for desiccating than wet samples. Final recovery, measured as a percentage of the initial level (before the treatment) was above 92%. Φ_{PSII} related to the effective transport mechanisms. The length and impact of the stress is more severe with slower and lower final recovery. Therefore, the difference between fully wet and desiccating samples was higher even though the total water loss at the end of the period was not high (1.25% of total water content) (*see* Fig. 2). Φ_{PSII} final recovery, after a 3 h relaxation period was 0.91% and 0.87% of initial values for wet and desiccating samples (*see* Fig. 3).

Even though the water loss at the end of the experimental period was not high, with 87.5% RWC (average of five samples with 0.02 standard deviation), there was apparent difference in NPQ time course in the fully wet and desiccating samples. The former presented a peak, right after the PI induction and followed by a relaxation of NPQ values during the three hours to a 154% of initial value. The NPQ decrease found in wet samples at the end of recovery period represented 79% of maximum found immediately after photoinhibitory treatment. The desiccating samples, however, kept the peak value during the whole relaxation period and slightly increased at the end of it (*see* Fig. 4).
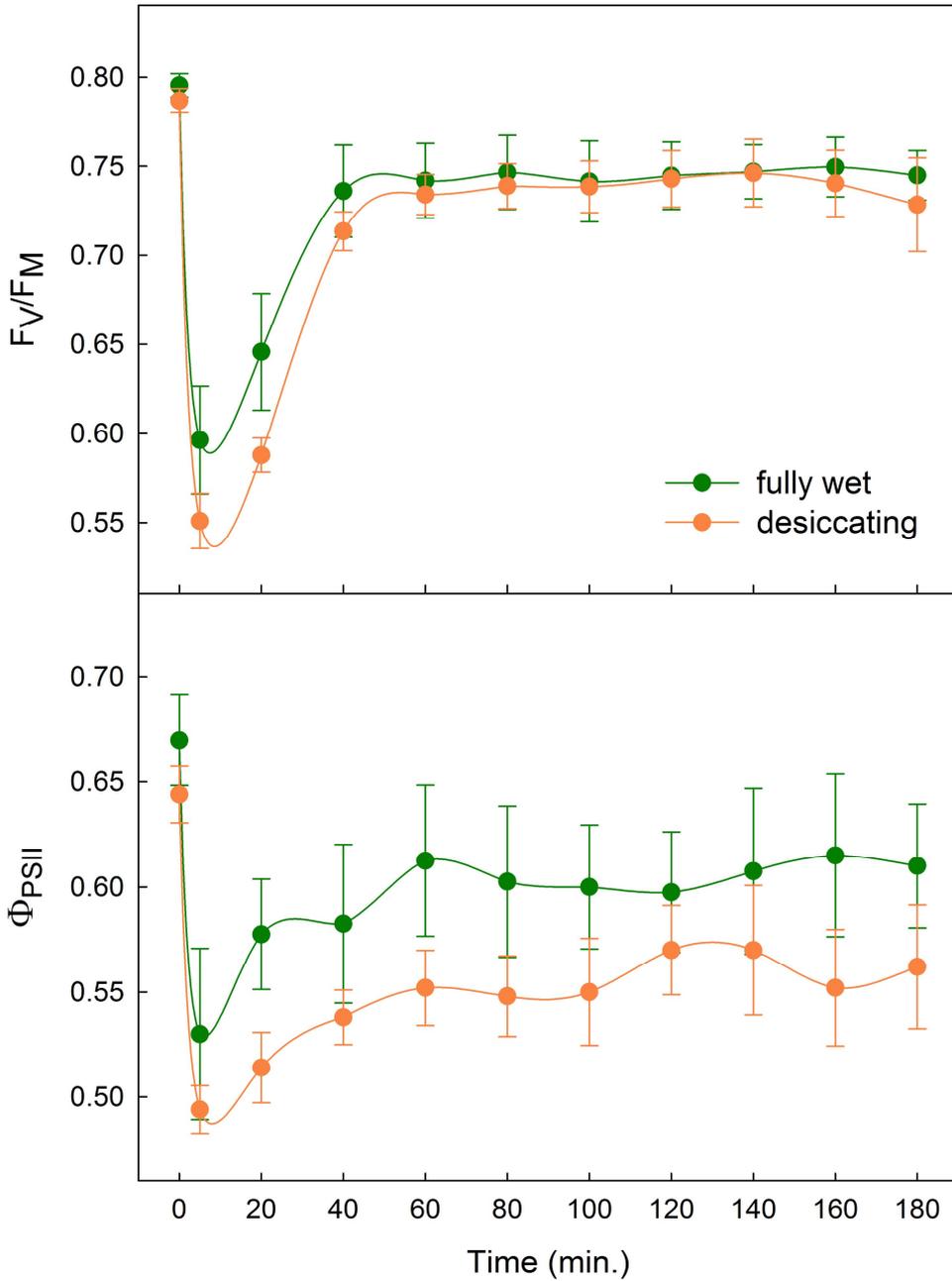
Fig. 2. F_V/F_M and Φ_{PSII} time courses, before (control time = 0) and during three hours after the photoinhibitory treatment in fully wet and desiccating samples.
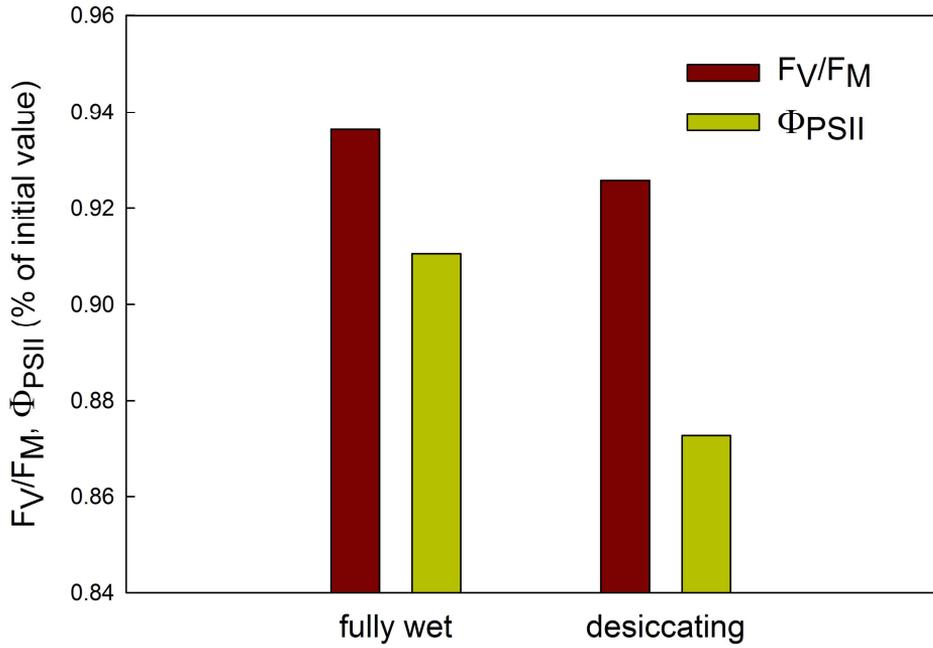
Fig. 3. F_V/F_M and Φ_{PSII} final recovery (%) of initial value in fully wet and desiccating samples.
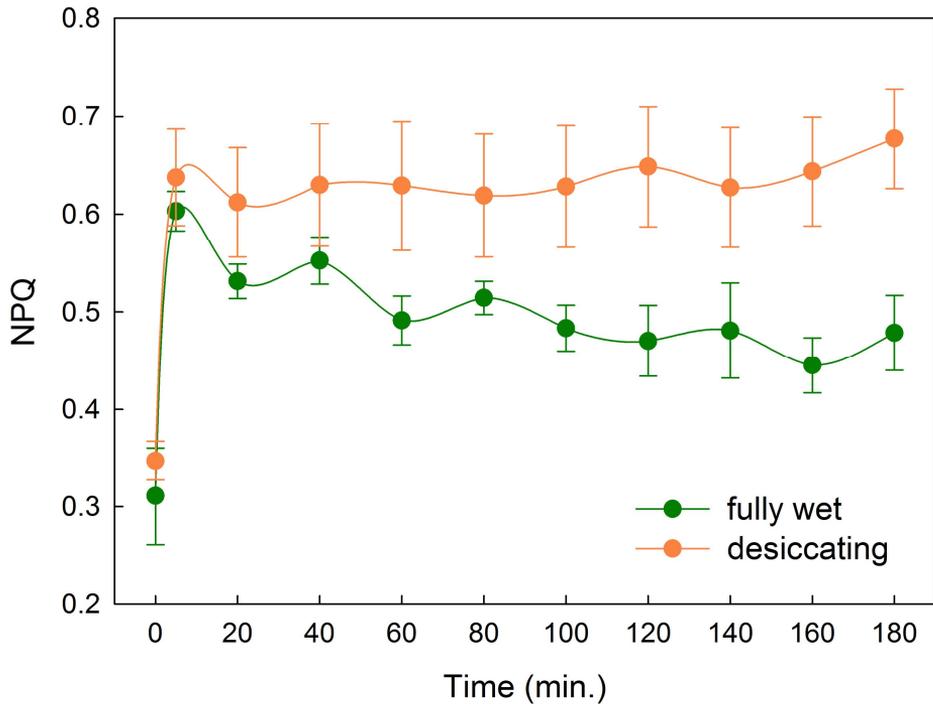

Fig. 4. NPQ time courses, before (control time = 0) and during three hours after the treatment in fully wet and desiccating samples.

Desiccation (PAM measurements)

Φ_{PSII} relation to RWC was more or less constant in the first part of the desiccation process (RWC range from 100% (wet) to 40%). Then, with pronounced desiccation, a significant decrease of Φ_{PSII} was apparent at the RWCs below 40% (Fig. 5). Such Φ_{PSII} decline indicated lowering of primary photosynthetic processes in PSII (for details *see* Discussion). Minimum Φ_{PSII} level (0.23) was recorded at 25% RWC (*see* Fig. 5). Non-photochemical quenching (qN, for equation, *see* Appendix) showed constant values with thallus desiccation till around 60% RWC. With further desiccation (RWCs below 60%) an increase in qN ended in maximum value of 1.0 reached at the RWC of 25%.
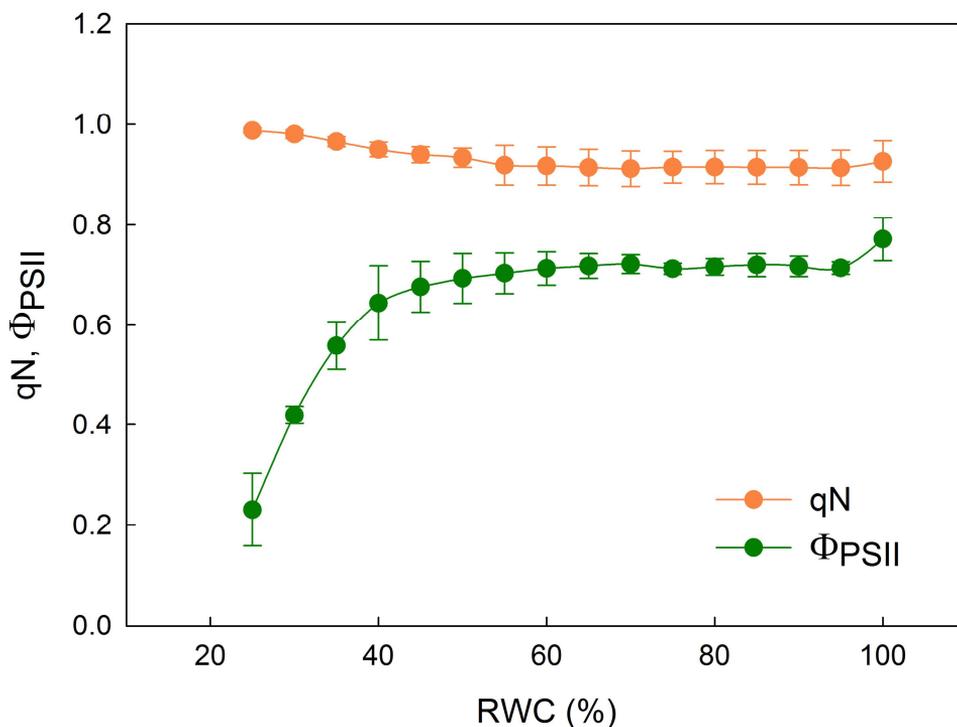

Fig. 5. qN and Φ_{PSII} response curve to relative water content (RWC). Error bars represent ± standard deviations.

Desiccation (OJIP measurements)

Majority of OJIP-derived chlorophyll fluorescence parameters (F_V/F_M, ABS/RC and DI_0/RC) showed the first sign of desiccation-induced changes at the RWC of 40% (Fig. 6). TR_0/RC and ET_0/RC started their increases at a higher RWC (around 70%). Their courses were of similar shape, only the TR_0/RC increase was found steeper at final phase of desiccation ET_0/RC (*see* Fig. 7). Performance index (Pi_ABS), which is considerd a general indicator of plant vitality, started to decrease at the RWC of 70%. Then, with following thallus desiccation from the RWC of 70% to 0%, Pi_ABS decreased at constant rate (Fig. 7).
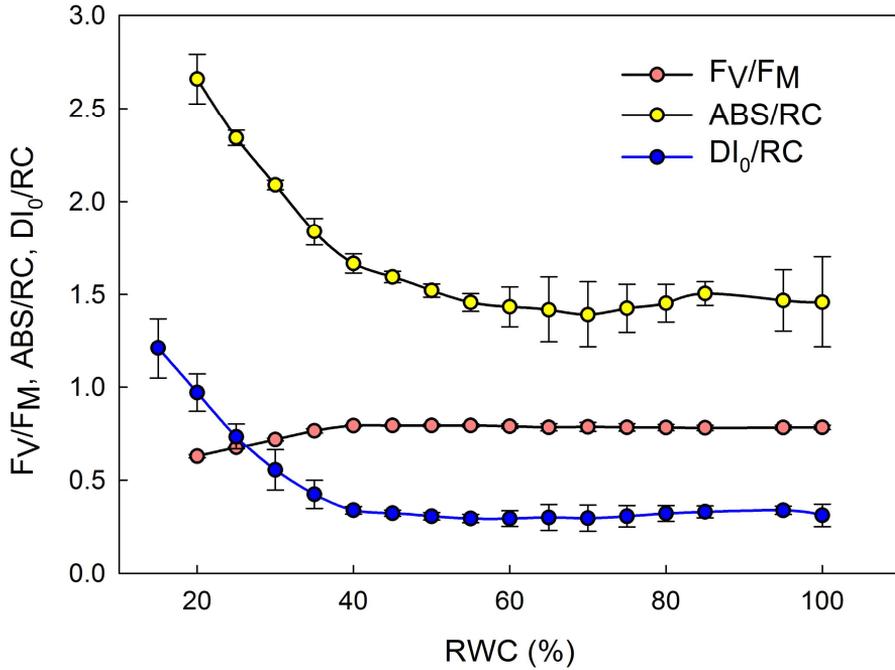
Fig. 6. F_V/F_M, ABS/RC and DI_0/RC response curve to relative water content (RWC). Error bars represent ± standard deviations.
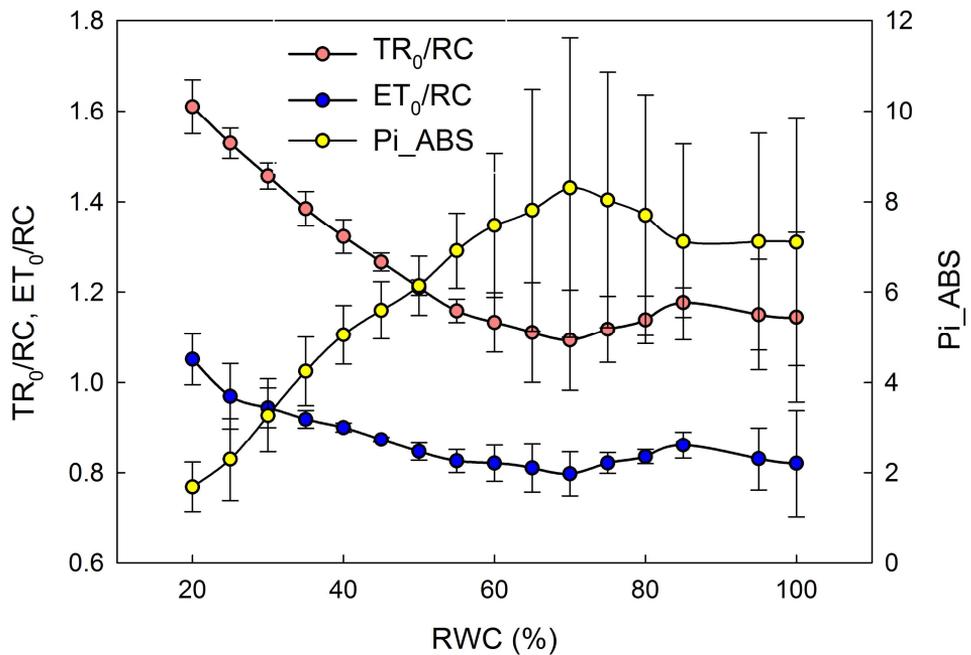

Fig. 7. TR_0/RC, ET_0/RC and Pi_ABS response curve to relative water content (RWC). Error bars represent ± standard deviations.

Discussion

Both stress factors were affecting the capacity of photosynthetic processes in PSII (F_V/F_M) and the efficiency of the electron transport utilized in the PSII photochemistry (Φ_{PSII}). Photoinhibitory treatment did not have a high impact on these parameters but the additional factor of desiccation (even in small extent) highly enhanced the photoinhibition-induced negative effects (*see* next paragraph). Relatively low effect of photoinhibitory treatment on F_V/F_M and Φ_{PSII} decrease, and fast recovery in wet *P. commune* samples are comparable with the data for *Sanionia uncinata* (Orekhova et al. 2021b). The study reports full recovery of F_V/F_M and Φ_{PSII} reached 90 min. after termination of the photoinhibitory treatment. These results support the idea that primary photosynthetic processes in well-hydrated *P. commune* are resistant to photoinhibition. The resistance is associated with protective mechanisms, particularly non-photochemical quenching and underlying mechanisms (for review *see* Robinson and Waterman 2014). Recently, several mechanisms associated with protein-associated quenching has been described for mosses (Alboresi et al. 2011, Gerotto et al. 2012). They comprise both PsbS and Light Harvesting Complex Stress-Related proteins (LHCSRs), whose expression is induced by high light stress. PsbS is associated mainly with the qE component of non-photochemical quenching (Niyogi et al. 2005). This is consistent with the qE increase witnessed in *P. commune* during the photoinhibitory treatment (data not shown). LHCSR1 protein is associated with thermal dissipation (Stella 2016 for *Physcomitrella patens*).

F_V/F_M derived from the OJIP curves recorded during *P. commune* desiccation, started to decrease when a part of the captured energy started to be dissipated as heat as indicated by the simultaneous increase of dissipated excitation energy,

DI_0/RC (*see* Fig. 6). This is consistent with the data measured by Orekhova et al. (2021b) who reported a photoinhibition-induced increase in DI_0/RC with consequent recovery in *S. uncinata*.

High resistance to water loss was apparent until 40% RWC value, placing this moss among the medium-to-high desiccation resistant mosses. This was clearly showed by the more or less constant value of Φ_{PSII} until 40% RWC and following Φ_{PSII} decline accompanied by an increase of qN at the same level of water content (*see* Fig. 5). In the same way, all parameters related to electron transport efficiency and thermal dissipation were showing substantial change at 40% RWC (*see* Figs. 6, 7), with the exception of Pi_ABS which was continuously decreasing (*see* Fig. 7). Being Pi_ABS an index of general energy transfer performance, this is consistent with all the other data from different measuring techniques, showing an impact on both light harvesting complexes and electron transport efficiency, induced by desiccation. The initial fluctuation of Pi_ABS curve could be attributed to the geometrical changes occurring in the first stage of desiccation mentioned above; inward folding of the leaf margins, along with the accompanying movements, could make the first measurements irregular.

The raise of ABS/RC could be interpreted as the result of the damage or destruction of some Chl molecules in the RCs, leaving behind the most efficient ones. Such response was described in poikilohydric autotrophs for other stressors such as *e.g.* high temperature (Marečková and Barták 2017). In the same way TR_0/RC and ET_0/RC (related to energy flux per RC, respectively leading to Q_A reduction, and photosynthetic linear electron transpor), increased at the final stages of desiccation, to cope with the excess of absorbed light to be transferred and conveyed on a smaller number of RCs than in hydrated state.

Similar increase in TR_0/RC and ET_0/RC has been described for desiccating lichen (Bednaříková et al. 2020).

Activation of non-photochemical quenching is a well-known regulatory mechanism to balance the absorption and utilization of light energy in order to minimize photooxidative damage; *see* Eberhard et al. (2008) and Li et al. (2009) for overall reviews. The data shown in Fig. 5 indicated constitutively high non-photochemical quenching (*i.e.* before the desiccation started). An increase found in qN at the RWC values below 40% might be associated with an increasing demand to quench absorbed light energy in less functioning PSIIs in severely dehydrated *P. commune* thallus. This is well comparable to the evidence reported for Antarcic mosses (*Brachythe-cium austroglareosum, Bryum pseudotriquetrum*) which tended to increase qN at the RWC values of 50 and 30%, respectively (Orekhova et al 2021b).

For desiccation-induced qN increase in mosses, several protective mechanisms are considered. For excess energy dissipation, three types of quenchers are reported (for review *see* Yamakawa et al. 2012): (i) protonation of a thylakoid proteins, (ii) thermal dissipation of energy from light harvesting complexes (Heber et al. 2006, Heber 2008), and (iii) reversible photo-accumulation of a chlorophyll radical in RCs of PSII. Heber (2012) reviewed thermal dissipation from LHC of PSII in desiccating mosses. He called these mechanisms the desiccation-induced quenching and attributed them to mosses.

Conclusions

Environmental stressors such as high light and desiccation by themselves lead to different decreases in both F_V/F_M and Φ_{PSII}. This is the effect of energy transformations occurring in the harvesting antennae complexes and in the linear electron transport chain from PSII to PSI. During dehydration, the effect is proportional to the loss of RWC, visible from around 55% of RWC but substantial from 40% RWC. Combined ChlF techniques can provide insights into the mechanisms activated during stress conditions, such as dissipation of excess absorbed energy through heat dissipation and conformational changes or structural changes in the light harvesting complexes. Combination of stressors could amplify the damages to the photosynthetic machinery more than as a simply additional effect. In the experiment, the quenching mechanisms were activated immediately when PI was induced, and after a high degree of water loss in the desiccation tests. In combined PI and desiccation treatment, quenching mechanisms were immediately activated and remained high for the whole experimental period. The degree of recovery and the relationship to water loss showed a good effectiveness of the protection. It must be noted that in the combined PI-desiccation experiment, the final water loss was too small to produce a severe impact in the involved parameters, so more experiments with increasing desiccation levels should be carried out.

References

ALBORESI, A., GEROTTO, C., CAZZANIGA, S., BASSI, R. and MOROSINOTTO, T. (2011): A red-shifted antenna protein associated with photosystem II in *Physcomitrella patens*. *The Journal of Biological Chemistry*, 286: 28978-28987.

BECKETT, R. P., MARSCHALL, M. and LAUFER, Z. (2005): Hardening enhances photoprotection in the moss *Atrichum androgynum* during rehydration by increasing fast- rather than slow-relaxing quenching. *Journal of Bryology*, 27: 7-12.

BEDNAŘÍKOVÁ, M., VÁCZI, P., LAZÁR, D. and BARTÁK, M. (2020): Photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum* when affected by desiccation and low temperatures. *Photosynthesis Research*, 145(3): 159-177.

CSINTALAN, Z., PROCTOR, M. C. F. and TUBA. Z. (1999): Chlorophyll fluorescence during drying and rehydration in the mosses *Rhytidiadelphus loreus* (Hedw.) Warnst., *Anomodon viticulosus* (Hedw.) Hook. & Tayl. and *Grimmia pulvinata* (Hedw.) Sm. *Annals of Botany*, 84: 235-244.

EBERHARD, S., FINAZZI, G. and WOLLMAN, F-A. (2008): The dynamics of photosynthesis. *Annual Review of Genetics*, 42: 463-515.

GEROTTO, C., ALBORESI, A., GIACOMETTI, G. M., BASSI, R. and MOROSINOTTO, T. (2012): Coexistence of plant and algal energy dissipation mechanisms in the moss *Physcomitrella patens*. *New Phytologist*, 196: 763-773.

GLIME, M.J. (2017): Photosynthesis: Photoinhibition. *In*: J. M. Glime (ed.): Bryophyte Ecology. Vol. 1, chapter 11-2-1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 11 November 2017. Available from http://digitalcommons.mtu.edu/bryophyte-ecology/.

HEBER, U., BILGER, W. and SHUVALOV, V. A. (2006): Thermal energy dissipation in reaction centres and in the antenna of photosystem II protects desiccated poikilohydric mosses against photooxidation. *Journal of Experimental Botany*, 57: 2993-3006.

HEBER, U. (2008): Photoprotection of green plants: A mechanism of ultra-fast thermal energy dissipation in desiccated lichens. *Planta*, 228(4): 641-650.

HEBER, U. (2012): Conservation and dissipation of light energy in desiccation-tolerant photoautotrophs, two sides of the same coin. *Photosynthesis Research*, 113: 5-13.

LI, Z., WAKAO, S., FISCHER, B. B. and NIYOGI, K. K. (2009): Sensing and responding to excess light. *Annual Review of Plant Biology*, 60: 239-260.

MAREČKOVÁ, M., BARTÁK, M. (2017): Short-term responses of primary processes in PS II to low temperature are sensitively indicated by fast chlorophyll fluorescence kinetics in Antarctic lichen *Dermatocarpon polyphyllizum*. *Czech Polar Reports*, 7: 74-82.

MAREČKOVÁ, M., BARTÁK, M. and HÁJEK, J. (2019): Temperature effects on photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum*: A chlorophyll fluorescence study. *Polar Biology*, 42: 685-701.

NABE, H., FUNABIKI, R., KASHINO, Y., KOIKE, H. and SATOH K. (2007): Responses to desiccation stress in bryophytes and an important role of dithiothreitol-insensitive non-photochemical quenching against photoinhibition in dehydrated states. *Plant and Cell Physiology*, 48: 1548-1557.

NIYOGI, K. K., LI, X.-P., ROSENBERG, V. and JUNG, H.-S. (2005): Is PsbS the site of non-photochemical quenching in photosynthesis? *Journal of Experimental Botany*, 56: 375-382.

OREKHOVA, A., BARTÁK, M., CASANOVA-KATNY, A. and HÁJEK, J. (2021a): Resistance of Antarctic moss *Sanionia uncinata* to photoinhibition: Chlorophyll fluorescence analysis of samples from the western and eastern coasts of the Antarctic Peninsula. *Plant Biology*, 23: 653-663. https://doi.org/10.1111/plb.13270.

OREKHOVA, A., BARTÁK, M., HÁJEK, J. and MORKUSOVÁ, J. (2021b): Species-specific responses to spectral reflectance and the photosynthetic characteristics in two selected Antarctic mosses to thallus desiccation. *Acta Physiologiae Plantarum*, accepted in press.

PERERA-CASTRO, A. V., WATERMAN, M. J., TURNBULL, J. D., ASHCROFT, M. B., MCKINLEY, E., WATLING, J. R., BRAMLEY-ALVES, J., CASANOVA-KATNY, A., ZUNIGA, G., FLEXAS, J. and ROBINSON, S. A. (2020): It is hot in the sun: antarctic mosses have high temperature optima for photosynthesis despite cold climate. *Frontiers in Plant Science*, 11: 1178.

PINNOLA, A., DALL'OSTO, L., GEROTTO, C., MOROSINOTTO, T., BASSI, R. and ALBORESI, A. (2013): Zeaxanthin binds to light-harvesting complex stress-related protein to enhance non-photochemical quenching in *Physcomitrella patens*. *The Plant Cell*, 25: 3519-3534.

ROBINSON, S. A., WATERMAN, M. J. (2014) Sunsafe bryophytes: Photoprotection from excess and damaging solar radiation. *In*: D. T. Hanson, S. K. Rice (eds.): Photosynthesis in Bryophytes and Early Land Plants, pp. 113–130.

STELLA, G. R. (2016): Light stress and photoprotection in green algae, mosses and diatoms. Ph.D. thesis, University of Verona and University Pierre et Marie Curie, 144 p.

STRASSER, R. J., TSIMILLI-MICHAEL, M. and SRIVASTAVA, A. (2004): Analysis of the chlorophyll *a* fluorescence transient. *In*: G. C. Papageorgiou, Govindjee (eds.): Chlorophyll *a* fluorescence: A signature of photosynthesis. Advances in Photosynthesis and Respiration. Springer, Dordrecht, the Netherlands, pp. 321–362.

TAKAHASHI, S., BADGER, M. R. (2011): Photoprotection in plants: A new light on photosystem II damage. *Trends in Plant Science*, 16: 53-60.

YAMAKAWA, H., FUKUSHIMA, Y., ITOH, S. and HEBER, U. (2012): Three different mechanisms of energy dissipation of a desiccation-tolerant moss serve one common purpose: To protect reaction centres against photo-oxidation. *Journal of Experimental Botany*, 63: 3765-3775.

Appendix (special formulae used)

$$NPQ = (F_M - F_M')/F_M'$$

$$qN = (F_M - F_M')/F_M$$

where

F_M is maximum chlorophyll fluorescence induced by saturation pulse on dark-adapted sample.

F_M' is maximum chlorophyll fluorescence induced by saturation pulse on light-adapted sample.